LC Troubleshooting

To Change or Not to Change?

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The column inlet frit is a common source of peak shape problems, but changing the frit risks disturbing the column packing.

The diagnosis and correction of chromatogram problems can be a daunting task in liquid chromatography (LC). However, several problem types produce "signature" chromatograms. Just as people can be identified by their unique signatures, these problems produce characteristic chromatograms that point to the problem source. A pair of these signature chromatograms is shown in Figure 1. When all the peaks in the chromatogram tail or show splitting, you should suspect immediately that a blocked frit or a column void is the problem source. In this month's "LC Troubleshooting," we'll look at how to correct this problem and at some practices that will help to prevent similar problems in the future.

THE OPTIONS

In my experience, blocked frits are much more common than column voids, but this certainly depends on the specific chromatographic conditions being used. The pressure may or may not be elevated. When you see the symptoms of the chromatograms in Figure 1 and suspect that a blocked frit is the cause, two procedures could correct the problem — frit replacement and column reversal. Either of these fixes correct the problem about a third of the time. Each procedure has advantages and disadvantages, and the choice is yours of which to try first.

Frit replacement: My preferred choice for column first aid is to replace the frit at the column head, although this is not a risk-free option. Frit replacement generally is a simple task, and it gives you a chance to examine the head of the column for voids; however, it risks disturbing the column packing bed and causing further column damage.

Before removing the column endfitting, be sure that the column is at room temperature. Then, hold the column vertically in a vise or ring-stand clamp to prevent unnecessary jarring of the column while you work on it. You should not hold the column in your hand when removing the frit, because the heat from your hand can cause the packing to expand and extrude from the column. And just like toothpaste from a toothpaste tube, the column packing will not go back into the column after it is out. If you must hold the column, minimize your contact with it by holding it on the endfitting, so that little heat will transfer to the column.

Use two wrenches to loosen the endfitting at the column inlet. (Columns with finger-tightened endfittings are often used for cartridge columns. These columns usually have pressed-in frits that are not user-serviceable, so try column reversal, which is discussed later.) When the fitting is loose, remove it carefully. If you are lucky, the frit will be lying loose on the top of the column, and you can remove it easily. More likely, however, the frit will remain lodged inside the column endfitting. Try one or more of the following techniques to dislodge the frit. Tap the endfitting (frit-side down) sharply on the lab bench; many times this will dislodge the frit. Sometimes you can blow the frit out with compressed air (be careful where you aim). Another option is to reconnect the fitting to the LC system and turn on the pump — if the frit is blocked, the increased pressure on the upstream side will often loosen or turn the frit in the fitting. At least one company sells a syringe-like device that screws into the narrow end of the fitting, allowing the plunger to pass through the fitting and push out the frit.

After the frit is loosened or turned, usually you can dig it out with a paper clip or spatula. If all your efforts at removing the frit fail, substitute an endfitting from an old column of the same brand. This adds additional risk, however, because unless the port depths of the two fittings are identical, the new frit may not seal properly.

After you have removed the frit successfully, the next step is examining the column packing bed. A new column will contain white packing material that should be level with the end of the column or slightly domed (<1 mm). If you have used the column enough that the frit is blocked, you will rarely see white packing. Instead, the packing will be gray, green, yellow, or some other color resulting from irreversible adsorption of pigments from your samples. Generally, this discoloration is not a problem and should be ignored. Don't try to remove the colored packing and replace it with fresh packing — it is more likely that you will damage the column than correct anything. If the column packing has settled below the rim of the column, or if you see any holes or channels in the column bed, the column has a void and should be discarded. Repairing voids is rarely a complete success — generally a lot of effort is expended trying to fix the column, and it often still performs unsatisfactorily after the attempted repair.

If the packing looks smooth and undisturbed, gently place a new frit on the top of the column. I recommend that you use a frit purchased from the column supplier, because it is very important that the frit is of the proper dimensions. A frit that is a little too thick will

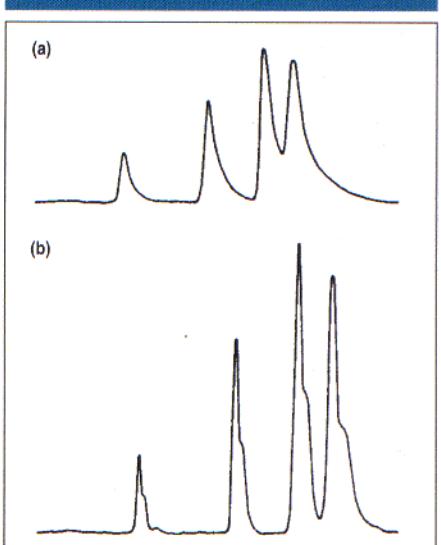

Figure 1: Problems caused by a column void or blocked frit, including (a) peak tailing and (b) peak splitting. (Courtesy of Thermo Separation Products, Fremont, California.)

generally be okay, but a thin frit might not tighten fully and can damage an otherwise good column. If you buy frits from third-party suppliers, be sure that the dimensions match the frit you removed from the column. After the frit is in place, carefully rinse and wipe the ferrule to remove any packing particles or other residue. Also rinse the endfitting thoroughly (some users like to sonicate the fitting). Tighten the fitting with your fingers, then complete the tightening with two wrenches. Be sure to tighten the fitting fully. As opposed to the 1/16-in. tubing connections, which are easily damaged by overtightening, it is difficult to overtighten the 1/4-in. column endfittings unless you are careless.

After the column is reassembled, connect the inlet end to your LC system and flush the column with 10 column volumes (typically 25 mL) of mobile phase before reconnecting the column outlet to the system.

Column reversal: An alternative to frit replacement is column reversal. The advantages of column reversal are that it is faster than frit replacement and noninvasive. Column reversal also can speed the removal of strongly retained materials from the old head of the column. Column reversal is the only way to clear a blocked frit if the frit is pressed into the end of the column, as is common with cartridge-column configurations. The main disadvantage of column reversal is that users cannot inspect the column inlet. If there is a void at the head of the column, you merely move the void to the outlet end where, depending on the conditions, the column packing may shift and cause further chromatographic problems.

Silica-based columns, such as the standard C18 and other bonded phases, generally can be reversed without consequence. Specialty columns, such as gel filtration or resin-based ion-exchange columns, may not be so robust. If you are uncertain of the stability of the reversed column, first check the literature that is shipped with the column, and if the answer is not obvious, call the manufacturer.

Column reversal is simple — just disconnect the column, reverse it, and connect the old outlet to the tubing from the injector. Pump about 20 column volumes of mobile phase (~50 mL for a 25 cm × 4.6 mm column) through the column to flush any particulates from the frit before connecting the outlet (old inlet) to the detector. Leave the column in this reverse-flow configuration and continue operation.

WERE YOU SUCCESSFUL?

If frit replacement or column reversal is successful, you should see some relief from any excessive pressure you observed. The peak shapes should return to normal, and you should be able to continue running samples. Remember, however, that either of these fixes is effective perhaps only a third of the time, so your efforts may be for naught. Also, if enough samples have been injected onto the column to block the frit, the column probably has been chemically stressed as well. This means that you may recover from the double peaking you observed, but the selectivity (peak spacing) could be inadequate for further work. At best, these corrective measures only extend the column life — they won't restore it to its original condition. So it is a good idea to have a spare column on hand, especially after performing minor column surgery.

PREVENTION

Problems with the column frit generally indicate more serious problems in the operation of the LC system. Any time you have to replace the frit, you are risking damage to a very expensive part of the system — the analytical column. It is more cost effective and easier to prevent these problems by using an in-line filter and guard column upstream from the column.

The in-line filter should be installed directly downstream from the injector or autosampler. The filter should contain a 0.5-µm porosity frit that will become blocked much more readily than a 2.0-µm frit at the head of the column. If the system pressure starts to rise, it is a good indication that the in-line filter is getting blocked. You can change this frit in a minute or two without disturbing the analytical column bed. In-line filters are called *zero-volume* filters because they have no practical effect on the dead volume of the system. I recommend that every LC system have an in-line filter installed, even if a guard column or other protective device is used.

A guard column should be used whenever the samples are likely to contain anything that can be irreversibly retained on the column (that is, most real samples). It should also be used whenever the mobile-phase pH is near the pH limits of the column (for example, above ~pH 7 for silica-based columns).

The guard column is a sacrificial element in the LC system. It catches particulate matter, binds irreversibly with strongly retained material, and withstands attacks by aggressive mobile phases. It is better that this damage strikes the inexpensive and disposable guard column rather than the expensive analytical column. Use a guard column that is designed for use with your particular brand and model of LC column — it is best to buy the guard column from the same supplier as the analytical column.

Replace the guard column whenever the combined performance of the guard column and the analytical column is worse than the analytical column alone. Observe the valley between two closely eluted peaks to check for guard column performance. Most workers replace the guard column on a time (for example, once a week) or per-sample (for example, every 100 samples) basis. A good rule of thumb is to replace the guard column when it has reached 80% of its estimated useful life. Replacement will help avoid contaminant breakthrough onto the analytical column.

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